REMARKS

The 35 USC §103(a) Rejections

Claims 1, 2, and 5-11 were rejected under 35 U.S.C. §103(a) as being unpatentable over **Brey** et al. (1992) in view of **Georgiou** et al. (1994) and further in view of **Haseltine** et al. (1991), **Kang** (1993) and **Rodman** (1997). This rejection is respectfully traversed.

The present invention relates to the induction of cellular and humoral anti-HIV-1 immune responses in animals using an attenuated bacterial host that expresses HIV-1 transactivating protein or reverse transcriptase.

The Examiner contends that **Brey** et al. is directed to an attenuated strain of enteroinvasive bacteria that express a peptide or protein related to an epitope of malaria parasites. The bacteria can induce protective immune response against malaria. **Georgiou** et al. disclose recombinant DNAs that are suitable for expressing heterologous antigen on the surface of an enteric microorganism. The DNA construct encodes fusion protein that comprises a Lpp signal sequence, a portion of OmpA membrane protein and a

heterologous antigen of interest. **Haseltine** et al., **Kang** and **Rodman** all disclose sequences for HIV-1 tat protein.

The Examiner concludes that "it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to express the HIV-1 tat gene provided by **Haseltine** et al. (1991), **Kang** (1993) or **Rodman** (1997) as an Lpp-OmpA-Tat fusion protein, as suggested by **Georgiou** et al. (1994), in the *S. typhimurium* expression system described by **Brey** et al. (1992), since **Brey** and colleagues teach that this system is useful for generating strong immune responses against the antigen of interest." Applicant respectfully disagrees.

The issue in dispute is whether the teaching of inducing protective a immune response against malaria by an attenuated strain of *Salmonella* expressing a peptide or protein related to an epitope of malaria parasites can be generalized to induction of cellular and humoral immune responses against HIV-1 Tat. As stated in the last response to Office Action, Applicant reiterated that the cited references do not provide one of ordinary skill in the art with the requisite expectation of successfully producing Applicant's claimed invention because induction of immune responses against HIV-1 Tat has to be determined empirically.

Induction of cellular and humoral immune responses against an antigen involves different cellular pathways. After an antigen is delivered to a cell by a delivery vehicle such as an attenuated strain of *Salmonella*, the antigen is broken down into fragments. Depending on the characteristics of individual fragment, some fragments may bind to MHC Class I molecule which would then present the antigenic fragments to T cells to induce cellular immune responses, whereas some other fragments may bind to MHC Class II molecule which would then present the antigenic fragments to B cells to induce humoral immune responses. Whether a putative antigen possesses antigenic fragments that would induce cellular and/or humoral immune responses has to be determined experimentally. One of ordinary skill in the art could not predict with any degree of reasonableness that an attenuated strain of *Salmonella* expressing any HIV-1 polypeptide would induce cellular and humoral anti-HIV-1 immune responses simply because antibody response against malaria was induced by the same bacteria expressing malarial antigens. The cited prior art does not teach any structural or biological relationship between malaria protein and HIV-1 transactivating protein or HIV-1 reverse transcriptase that would enable one of ordinary skill in the art to predict these

proteins would induce similar immune responses when they are delivered by an attenuated strain of *Salmonella*. The state of the art is that induction of cellular and/or humoral immune responses by a putative antigen cannot be predicated and ascertained until actual experiments are carried out in model animals. In the absence of teaching related to inducing specific immune responses to HIV-1 Tat or reverse transcriptase, the cited references do not provide one of ordinary skill in the art with the requisite expectation of successfully producing Applicant's claimed invention.

The Examiner contends that "the prior art clearly illustrates that attenuated *Salmonella* expression vectors are useful for generating strong humoral and cellular immune response against a heterologous antigen when said antigen is expressed on the bacterial surface, preferably as a stable lpp-OmpA-fusion protein." Applicant submits that this point is not supported by the prior art.

Applicant would like to draw the Examiner's attention to the distinction between attenuated *Salmonella* expression vectors as a delivery vehicle verse induction of humoral and cellular immune responses. Although the prior art teaches the usefulness of attenuated *Salmonella* expression vectors as a delivery vehicle, induction of immune responses against a heterologous antigen

depends solely on the property of the heterologous antigen itself. As discussed above, induction of humoral and cellular immune responses depends on whether the putative antigen possesses epitopes that can bind to and be presented by MHC Class I and Class II molecules. Induction of immune responses *in vivo* is a complex biological process the outcome of which must be determined experimentally.

The Examiner also contends that "one of ordinary skill in the art would expect a heterologous antigen, absent evidence to the contrary, to generate a strong immune responses in any given host when the recombinant *Salmonella* vaccine vector is administered. This is totally consistent with the teachings of the prior art." This assertion is not supported by the prior art.

Applicant reiterates that induction of immune responses (let alone strong immune responses) *in vivo* has to be determined by experimentation in an appropriate animal model. Without actual experimentation, one of ordinary skill in the art simply does not have any reasonable basis to predict or expect whether there would be induction of immune responses. To predict without any rational basis is just pure speculation; there is no reason for "reasonable" expectation.

Moreover, the Examiner's assertion that any heterologous antigen would induce strong immune responses in any given host when the recombinant *Salmonella* vaccine vector is administered is an overtly broad statement not supported by the prior art. One of ordinary skill in the art would not generalize findings regarding induction of immune responses by malaria antigen to any other heterologous antigens in any given host. One of ordinary skill in the art simply has to address these issues by experimentation.

Teaching in the prior art does not support the Examiner's assertion of "any heterologous antigen .... strong immune responses in any given host." **Hone** et al. (1996) teach that expression of gp120 in the cytoplasm of recombinant *Salmonella* "did not stimulate the development of gp120-specific serum IgG or cytotoxic T lymphocytes (CTLs)" (see abstract). In contrast, *Salmonella* bacteria expressing intracellular beta-galactosidase can induce anti-beta-galactosidase antibodies (**Brey** et al., column 6, lines 55-61). Moreover, although *Salmonella* bacteria expressing the B subunit of heat labile toxin (LT-B) induced mucosal anti-LT-B IgA, the antibody response induced by the recombinant *Salmonella* was significantly less than that induced by purified LT-B (**Brey** et al., column 6, lines

26-35). These results clearly indicate that one of ordinary skill in the art cannot extrapolate results from one antigen to any heterologous antigen generating strong immune responses in any given host. There are a number of variables such as humoral vs. cellular immune response, intracellular vs. cell surface location, and the nature and characteristics of the antigen itself. The state of the art does not provide generalization across the board. One of skill in the art must resolve these issues by actual experimentation.

The Examiner argues that "all of the components employed by the applicants e.g., attenuated bacterial host, surface exposure fusion antigen, and viral transactivating protein were well-known in the prior art. Both the bacterial host and fusion protein had already been used to produce recombinant proteins. Moreover, viral transactivating proteins have been cloned, sequenced an expressed in disparate expression systems. Therefore, there was a reasonable expectation of success of sufficient motivation for combining the aforementioned references." Apparently, the Examiner is arguing that a person having ordinary skill in this art would have reasonably expected successful expression of the viral transactivating protein as a fusion protein in the disclosed bacterial host.

The present invention, however, is not drawn to such bacteria. The essence of the present invention is the induction of cellular and humoral immune responses against HIV-1 by a recombinant bacterial host expressing HIV-1 transactivating protein or reverse transcriptase. Even though it is obvious to try and construct an HIV-1 antigen-expressing bacterial host, it is not obvious from the combined teaching of **Brey** et al., **Georgiou** et al., **Haseltine** et al., **Kang** and **Rodman** that such bacteria can induce both cellular and humoral anti-HIV immune responses as claimed herein. The cited references do not teach or suggest methods or reagents that can induce cellular and humoral immune responses against HIV-1 transactivating protein or reverse transcriptase. As discussed above, absent *in vivo* experiments related to the specific anti-HIV-1 immune responses, the cited references fail to provide the requisite expectation of successfully producing Applicant's claimed invention. The invention as a whole was not obvious to one of ordinary skill in the art when the invention was made.

Claims 1, 2, 5 and 11 have been canceled. Claims 12 and 13 are added to incorporate the properties of the bacterial host recited in the deleted claims. Applicant submits that no new matter

has been added. Accordingly, Applicant respectfully request that the rejection of claims 6-10 under 35 U.S.C. §103(a) be withdrawn.

Claims 1, 2, and 5-11 were rejected under 35 U.S.C. §103(a) as being unpatentable over **Hone** et al. (1996) in view of **Georgiou** et al. (1994) and further in view of **Haseltine** et al. (1991), **Kang** (1993) and **Rodman** (1997). This rejection is respectfully traversed.

Hone disclosed induction of humoral immune response by an attenuated *Salmonella* vaccine vector expressing HIV-1 gp120 fusion protein. Other references have been discussed above.

The Examiner contends that "it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to express the HIV-1 tat gene provided by **Haseltine** et al. (1991), **Kang** (1993) or **Rodman** (1997) as an Lpp-OmpA-Tat fusion protein, as suggested by **Georgiou** et al. (1994), in the *S. typhimurium* expression system described by **Hone** et al. (1996), since **Hone** and colleagues teach that this system is useful for generating strong immune responses against the antigen of interest." Applicant respectfully disagrees.

The issue here is whether the teaching of inducing humoral immune response against HIV-1 gp120 by an attenuated

strain of *Salmonella* expressing HIV-1 gp120 fusion protein can be generalized to induction of cellular and humoral immune responses against HIV-1 transactivating protein or reverse transcriptase. As discussed above, Applicant reiterated that the cited references do not provided one of ordinary skill in the art with the requisite expectation of successfully producing Applicant's claimed invention because induction of immune responses, whether it is cellular or humoral, strong or weak, against a specific HIV-1 antigen has to be determined empirically.

The Examiner argues that the teaching of **Hone** et al. "support the proposal that *Salmonella* vectors will be a safe and inexpensive means for delivery of HIV antigens to, and the elucidation of HIV-specific T cells in the mucosal and systemic compartments" and "it is reasonable to propose, therefore, that *Salmonella* bearing surface-expressed rgp120 will elicit gp120-specific $CD8^+$ CTLs." The Examiner concludes that "there is a reasonable expectation that strong humoral and cell-mediated immune responses will be generated against HIV-1 antigens expressed in this system." Applicant respectfully disagrees.

As discussed above, although *Salmonella* vectors are useful delivery vehicles, induction of specific immune responses is a

property of the antigen. Even if gp120 can induce CTL response, one of ordinary skill in the art would not generalize the finding to other HIV antigens absent teaching that shows structural or biological relationship between gp120 and other HIV-1 antigens. After all, the current state of the art requires *in vivo* experiments to address the issue of immunogenicity of a particular antigen.

Furthermore, **Hone** et al. only show anti-gp120 antibody responses. **Hone** et al. do not show any data on anti-gp120 T cell responses. **Hone** et al. only speculate on anti-gp120 T cell response based on the results of anti-gp120 antibody responses. T cell response and antibody response, however, are two different arms of the immune system. T cell response is MHC Class I-restricted, whereas antibody response is MHC Class II-restricted. These two responses entail different antigen presentation pathways, and require different antigenic epitopes, even from the same antigen. **Hone** et al. do not provide any reasonable basis for predicting MHC Class I-restricted immune response based on MHC Class II-restricted response; it is only pure speculation without any reason for "reasonable" expectation of success.

The Examiner ignores **Hone's** teaching that the issue of inducing cellular anti-HIV-1 immune responses by *Salmonella*

vaccine strain is unresolved. **Hone** et al. teach that "presently there is no consensus on the vector configuration that optimizes the ability of *Salmonella* to induce foreign antigen-specific cytotoxic $CD8^+$ CTLs in vivo." (page 206, left column, third paragraph). In view of the fact that *Salmonella* vector expressing cytoplasmic gp120 (**Hone** et al.) or cytoplasmic influenza antigen cannot induce CTL response (**Hone** et al., page 206, left column, last line to right column, line 3), **Hone** et al. speculate that surface-expressed gp120 may induce gp120-specific CTLs. The important issue, however, is not surface location. A surface-expressed antigen may or may not induce cell-mediated immune response.

The critical issue is whether the antigen of interest expresses Class I MHC-restricted epitopes capable of inducing CTLs. If the antigen does not possess Class I MHC-restricted epitopes, no CTL will be induced even though the antigen is expressed on cell surface. Applicant reiterates that the issue of immunogenicity, i.e., whether there are MHC Class I/II-restricted epitopes, can only be resolved by experiments in animal models.

The Examiner also disregards Applicant's Declaration that shows attenuated *Salmonella* expressing HIV-1 reverse transcriptase epitopes induce cytotoxic $CD8^+$ T cell response. The

Examiner dismisses the results as not unexpected. However, as discussed above, there is no reasonable expectation without actual experimentation. Applicant submits that the Examiner has fallen victim to the insidious effect of a hindsight syndrome wherein what the invention taught is used against its teacher.

Similar to the rejection based on **Brey** et al. and other references, the Examiner argues that **Hone** et al. and other references provide reasonable expectation of success because various components of the instant invention have been disclosed. Applicant reiterates that obvious to try is not the standard of 35 U.S.C. §103. The essence of the present invention is the induction of cellular and humoral immune responses against HIV-1 by a recombinant bacterial host expressing HIV-1 transactivating protein or reverse transcriptase. Even though it is obvious to try and construct an HIV-1 antigen-expressing bacterial host, it is not obvious from the combined teaching of **Hone** et al., **Georgiou** et al., **Haseltine** et al., **Kang** and **Rodman** that such bacteria can induce both cellular and humoral anti-HIV immune responses as claimed herein. As discussed above, absent *in vivo* experiments related to the specific anti-HIV-1 immune responses, the cited references fail to provide the requisite expectation of successfully

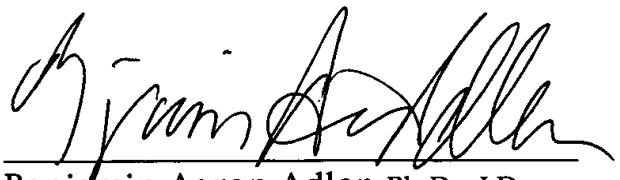
producing Applicant's claimed invention. The invention as a whole is not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 1, 2, 5 and 11 have been canceled. Claims 12 and 13 are added to incorporate the properties of the bacterial host recited in the deleted claims. Applicant submits that no new matter has been added. Accordingly, Applicant respectfully request that the rejection of claims 6-10 under 35 U.S.C. §103(a) be withdrawn.

This is intended to be a complete response to the Final Office Action mailed June 3, 2003. If any issues remain, the please telephone the undersigned attorney of record for resolution.

Respectfully submitted,

Date: Dec 3, 2003



Benjamin Aaron Adler, Ph.D., J.D.
Registration No. 35,423
Counsel for Applicant

ADLER & ASSOCIATES
8011 Candle Lane
Houston, Texas 77071
(713) 270-5391 (tel.)
(713) 270-5361 (facs.)
badler1@houston.rr.com